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Adenocarcinoma of Duodenum and Ampulla of Vater: Clinicopathology Study and Expression of p53, c-neu, TGF-α, CEA, and EMA

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Oncogenes, tumor suppressor genes, and growth factors are being explored as to their role in the initiation and progression of most neoplasms, but little information exists on the expression of oncoproteins or growth factors in adenocarcinoma of the duodenum or ampulla of Vater. This report covers expressions of p53, c-neu, TGF-α, CEA, and EMA in duodenal adenocarcinoma and ampullary adenocarcinoma, as well as correlations between expressions and tumor stage, histological grade and patient survival. The expression of p53, c-neu, TGF-α, CEA, and EMA has been studied in 15 duodenal adenocarcinomas and in eight ampullary adenocarcinomas by avidin-biotin-peroxidase complex indirect immunoperoxidase technique. The positive reaction for p53, c-neu, TGF-α, CEA, and EMA in duodenal adenocarcinoma was 20%, 60%, 60%, 73%, and 100%, respectively, and in ampullary adenocarcinoma, 13%, 100%, 50%, 63%, and 100%. Among the duodenal tumors, C-neu and p53 expression was noted more frequently in groups with high histological grades. Patients with c-neu positive duodenal adenocarcinoma had a shorter survival than the patients with c-neu negative duodenal adenocarcinoma ($P < 0.01$). C-neu product may serve as an unfavorable prognostic indicator in duodenal adenocarcinoma. No *statistically* significant correlation was found between the expressions of CEA, EMA, p53, and TGF-α and patient survival, tumor stage, or histological grade in either duodenal or ampullary adenocarcinomas.

KEY WORDS: **duodenal adenocarcinoma, ampullary adenocarcinoma, immunohistochemistry, p53, c-neu, TGF-α, CEA, EMA, survival**

INTRODUCTION

Adenocarcinomas of the duodenum and ampulla of Vater are uncommon neoplasms [1–3], representing <2% of all malignant tumors of the intestinal tract. Although oncogenes, tumor suppressor genes, and growth factors are being explored as to their role in the initiation and progression of most neoplasms, little information exists on the expression of oncoproteins or growth factors in adenocarcinoma of the duodenum or ampulla of Vater. In order to gain better understanding of the characteristics of duodenal and ampullary adenocarcinomas, the role of the oncogene, tumor suppressor gene, and growth factor in human adenocarcinomas of duodenum and ampulla of Vater and their value as prognostic indicators in duodenal and ampullary adenocarcinomas, we have studied the

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clinical findings in 21 patients with duodenal adenocarcinoma and 13 patients with ampullary adenocarcinoma and determined the expression of oncogene protein c-neu, suppressor gene protein p53, transforming growth factor alpha (TGF-α), carcinoembryonic antigen (CEA), and epithelium membrane antigen (EMA) in 15 of the former and in eight of the latter. Comparison was then made between the respective immunochemical expressions and the clinicopathological parameters (tumor stage, histological grade, and patient survival).

MATERIALS AND METHODS

Patients

Of an overall of 21 patients with duodenal adenocarcinoma, 15 were men and six were women. The ages ranged from 43 to 88 years (mean: 65 yr). Fifteen patients underwent tumor resection; six did not. The 13 patients with adenocarcinoma of ampulla of Vater included nine men and four women; ages ranging from 43 to 70 years (mean: 58 yr). Eleven patients had tumors resected; two did not. Vomiting, anemia, epigastric pain, jaundice, and weight loss were the common manifestations of both tumors. Most tumors were nodular, some with ulceration, and some were circumferential around the duodenal lumen. The greatest diameter of the tumors ranged from 1 cm to 15 cm. Operative treatment alone was employed until recurrent disease was detected. Follow-up data were obtained from the tumor registries plus the respective physicians' records.

Histological Grade

Morphological examination and histologic grading were carried out first independently and then collaboratively by two pathologists. Histological slides were available for secondary review in 27 cases (18 duodenal, 9 ampullary adenocarcinomas). Semiquantitative grading of each tumor was performed as follows: *(A) gland formation and architecture* (1: well-formed glands, 2: irregular and incomplete glands, and 3: poorly formed glands and nests); *(B) nuclear features* (1: most nuclei are regular, 2: most nuclei are slightly irregular, 3: nuclei are extremely irregular); and *(C) mitotic figures* (1: <1 mitosis per one high power field, 2: 2–3 mitoses per high power field, 3: >3 mitoses per high power field). Four grades (I–IV) of differentiation were then determined by adding the scores given in each of the three categories. Grade I (best differentiation) thus equaled a score of 3, Grade II = 4 or 5, Grade III = 6 or 7, and Grade IV = 8 or 9.

Tumor Staging

All patients were assigned a pathologic stage of disease according to the criteria of the American Joint Committee on Cancer's manual [4].

Antibodies Utilized

PAb 1801 (P53 Ab-2, Oncogene Science, Manhasset, NY) is a human-specific antibody that recognizes an epitope in p53 between amino acids 32-79. The dilution used was 1:120.

3B5 (c-neu Ab-3, Oncogene Science) is a monoclonal antibody raised against a synthetic peptide comprising amino acid residues 1242–1255 of the predicted sequences of the neu oncogene product. The dilution was 1:80.

The antibody 213–4.41 (TGF-α Ab-2, Oncogene Science) reacts with denatured and native TGF-α of human and rat origin. The dilution was 1:20.

Anti-CEA (DAKO, Carpinteria, CA) is a rabbit polyclonal anti-human carcinoembryonic antigen antibody. The working dilution was 1:2,000.

E29 (EMA Ab, DAKO) is a mouse monoclonal antibody reacting with epithelial membrane antigen with a molecular weight in the range of 265–400KD. The working dilution was 1:40.

Immunohistochemistry

The avidin-biotin-peroxidase complex indirect immunoperoxidase technique was performed on 5-μm sections from formalin-fixed, paraffin-embedded tissue. Before incubating with blocking serum, those slides that would react with p53 antibody were treated with 1 mM citric acid, pH 6.0, in a microwave oven three times for 5 minutes each and then cooled for 30 minutes. Negative controls omitted the primary antibodies. For CEA, EMA, and c-neu positive controls, respective known positive tissues were used; for TGF-α, a normal duodenal mucosa was used as positive control. SV-80 cell line was used as a p53 positive control.

Immunohistochemical analysis of each tumor was based not only on the grading of staining intensity (i.e., ±, +, ++), but also on the percentage of tumor cells that stained positive. If the staining intensity was "+" or greater and the percentage of positive cells in the slide was >5%, this tumor was considered to be expressing this protein. The cellular location of positive staining was also examined, e.g., cytoplasmic membrane staining, cytoplasm staining or nuclear staining.

Statistics

The Chi-square test was used to determine statistical significance between expressions and tumor stage, histological grade and patient's survival. *P* values are based on the one-tailed probability test.

RESULTS

Clinicopathological Findings

The tumor stage and histopathological grade are shown in Table I. Five-year survival for patients who underwent

TABLE I. Tumor Stage and Histopathological Grade

	Stage[a]				Grade			
	I	II	III	IV	I	II	III	IV
Duodenal carcinoma (n = 18)	2	9	4	2	5	5	4	4
Ampullary carcinoma (n = 9)	2	2	3	2	2	2	4	1

[a] Unclassified in one duodenal carcinoma.

tumor resection was 27% and 40%, respectively, in the duodenal and ampullary adenocarcinoma groups. No patient with unresectable tumor survived to 5 years in either duodenal or ampullary group. Five-year survival for patients for stage I, II, III, and IV duodenal adenocarcinoma was 100%, 22%, 0%, and 0% respectively, and for patients with ampullary adenocarcinoma was 100%, 100%, 33%, and 0%.

Immunohistochemical Findings

The expression at the protein level of EMA, CEA, p53, c-neu, and TGF-α as defined immunohistochemically was analyzed and the relationship between proteins and clinical and biological characteristics was correlated in 15 patients with adenocarcinoma of duodenum and in eight with ampullary adenocarcinoma. In Figure 1, expression patterns of EMA, CEA, p53, c-neu, and TGF-α are shown. The respective positive staining patterns were located within the cytoplasmic membrane (EMA), cytoplasm and luminal surface of the gland (CEA), nuclei (p53), cytoplasmic membrane and cytoplasm (c-neu), and cytoplasm (TGF-α). In a few cases (three duodenal carcinomas and one ampullary carcinoma), a TGF-α positive reaction was found not only in cytoplasm, but also in the nuclei of carcinoma cells, as well as in some of the normal epithelial cells of the same specimens. P53 and CEA expressions were not detectable by immunohistochemistry in normal duodenal mucosa, but were detected in adenocarcinoma cells. TGF-α and EMA expressions were detected in both benign and malignant epithelium of the duodenum. C-neu was expressed weakly in normal duodenal surface epithelium and glands, and was expressed strongly in the adenocarcinoma.

As shown in Table II, positive reactions for EMA, CEA, p53, c-neu, and TGF-α in duodenal adenocarcinoma were 100%, 73%, 20%, 60%, and 60%, respectively, and for ampullary tumors were 100%, 63%, 13%, 100%, and 50%. There was a tendency for c-neu expression to be detected more frequently in ampullary carcinoma (100%) as compared to duodenal carcinoma (60%). Table II also shows the relationship between expression and histological grade. All Grade I duodenal and ampullary tumors were p53 negative, *although there was no other significant relationship noted between p53 expression and histological grade or tumor's stage.* In duodenal adenocarcinoma, the expression of c-neu was detected more frequently in high histological grade groups (67% in Grade III, 100% in Grade IV) as compared to low grade groups (0% in Grade I, 60% in Grade II).

Twelve patients with adenocarcinoma of the duodenum studied immunohistochemically, underwent tumor resection; all three patients with p53 positive tumors died within 18 months after resection, whereas among those patients with p53 negative tumors, 44% survived over 18 months. All eight patients with c-neu positive tumors died within 18 months after resection, only one with c-neu negative tumor died within 18 months (Fig. 2). Differences in staging between the above subjects could not explain the differences in outcomes. The patients with c-neu positive duodenal adenocarcinoma had a shorter survival than patients with c-neu negative duodenal carcinoma ($P < 0.01$).

DISCUSSION

Michelassi et al. [5] reviewed 647 patients with tumors in the general periampullary area diagnosed during a period of 40 years. Among the 647 patients, 16 had duodenal and 28 had ampullary adenocarcinomas [5]. The 5-year survival rates among patients with duodenal adenocarcinoma and ampullary adenocarcinoma have been reported to be 20–30% and 30–60%, respectively [3,5–7]. In our patients, the 5-year survival rates were 27% and 40% after resection for duodenal and ampullary adenocarcinomas, respectively.

As an epithelial marker, EMA was expressed in all adenocarcinomas of duodenum and ampulla of Vater in the present study. Carcinoembryonic antigen (CEA) is expressed by endodermal tissues during the first two trimesters of embryonic and fetal development. It is also expressed by carcinomas of gastrointestinal origin and, to a lesser degree, in epithelial malignancies of different origins, such as the pancreas, lung, breast, ovary, and thyroid. Since CEA expression in normal adult tissue is negligible, it is the most widely used tumor marker in clinical practice. In our series, 73% duodenal and 63% ampullary adenocarcinomas expressed CEA. There was no significant relationship between CEA expression and tumor stage, histological grade, or survival with either cancer.

The human p53 gene is located on chromosome 17P. The wild-type gene, encoding a 53KDα nuclear phosphoprotein, may function as a tumor suppressor gene that

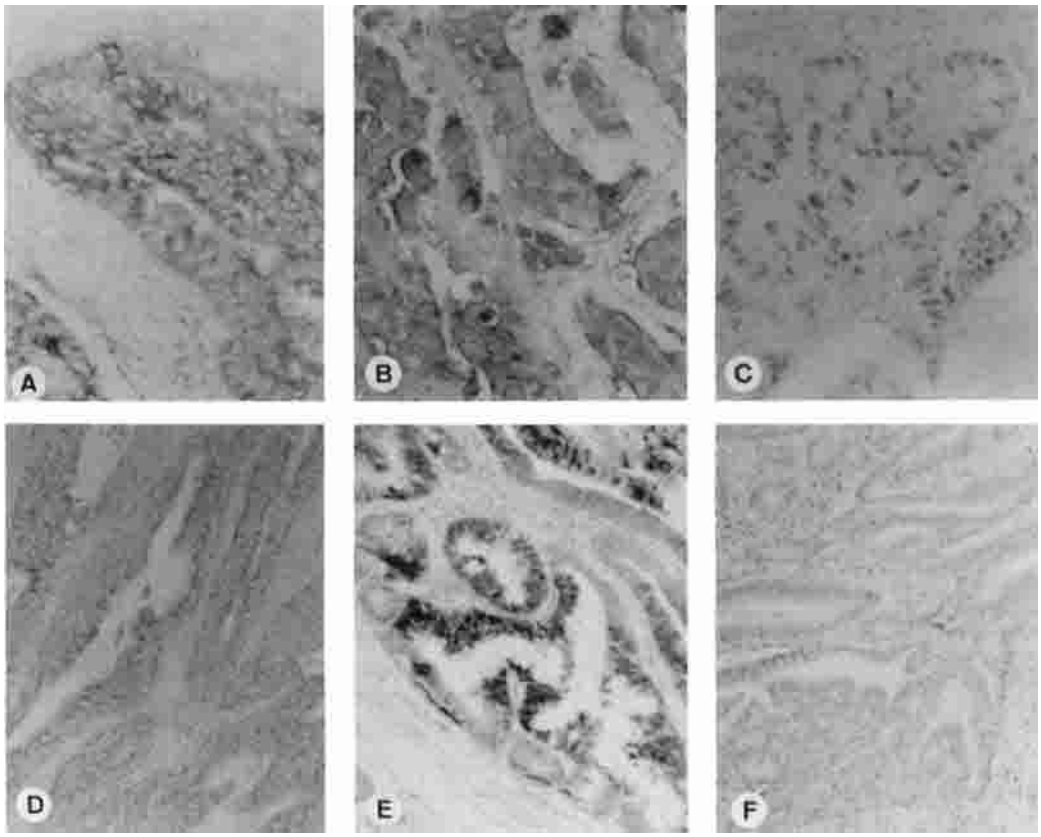

Fig. 1. The avidin-biotin-peroxidase complex indirect immunoperoxidase technique was performed on 5-μm sections from formalin-fixed, paraffin-embedded tissue. **A.** Ampullary adenocarcinoma (grade II, stage II) stained for EMA. Tumor cells show expression at plasma membrase (×200). **B.** Duodenal adenocarcinoma (grade III, stage II) stained for CEA. Positive reaction located at luminal surface of gland (×200). **C.** Duodenal adenocarcinoma (grade II, stage III), malignant nuclei are stained positive for p53 protein (×200). **D.** Duodenal adenocarcinoma (grade III, stage III) stained for c-neu. Cytoplasmic membrane staining is observed in tumor cells (×100). **E.** Ampullary adenocarcinoma (grade II, stage II) stained for TGF-α. Positive reaction located within cytoplasm. **F.** Negative control, omitting the primary antibody (×100).

regulates and constrains cell growth and division. It has been suggested that the loss of wild-type p53 function may play a role in malignant transformation. This loss may occur either by mutation of one copy of the p53 gene, followed by deletion of the remaining wild-type allele, or through inactivation of the wild-type protein after dimerization with the more stable mutant protein. In normal cells, p53 exhibits a very short half-life (5–20 min) and is thus undetectable by standard immunohistochemical methods [8,9]. In contrast, tumor cells with genetic alterations of p53 frequently have been found to express a more stable p53 protein that can be detected by immunohistochemistry. To date, the abnormalities of the p53 gene appear to be one of the most common gene alteration identified in carcinoma. In the literature, p53 expression has been detected from 35.6% to 45.5% in breast cancer [10,11], 35–55% in colorectal cancer [11–13], 43–65% in lung cancer [14,15], and 75% in pancreatic carcinoma [16]. In the present study, we have confirmed the expression of p53 in 20% of duodenal adenocarcinomas and in 13% of ampullary adenocarcinomas. Several investigators have indicated a correlation between p53 expression and patient survival [15,17], whereas others have not [10,12,14]. Our patients with p53 positive duodenal adenocarcinoma died within 18 months after operation, in contrast to those patients with p53 negative duodenal adenocarcinoma whose survival rate at 18 months was 44%, although there was no statistically significant relationship between p53 expression and survival time.

The c-neu oncogene, which belongs to the tyrosine kinase oncogene family and codes for 185-KDα glycoprotein, has been suggested as being involved in the pathogenesis of human cancer. The expression of c-neu protein determined by immunohistochemistry has been observed in a number of normal and neoplastic human tissues including breast, ovary, lung, pancreas, ureter, stomach, small intestine, colon, and kidney [11,14,18,19–23]. Previous studies on pancreatic, ovarian, and breast cancer have suggested that c-neu expression is associated

TABLE II. Relationship of Histological Grade and Antigen Expression

A. Duodenal carcinoma Grade		EMA(%)	CEA(%)	P53(%)	C-neu(%)	TGF-a(%)
I	(n = 3)	3 (100)	1 (33)	0 (0)	0 (0)	2 (67)
II	(n = 5)	5 (100)	4 (80)	1 (20)	3 (60)	3 (60)
III	(n = 3)	3 (100)	3 (100)	1 (33)	2 (67)	2 (67)
IV	(n = 4)	4 (100)	3 (75)	1 (25)	4 (100)	2 (50)
Total	(n = 15)	15 (100)	11 (73)	3 (20)	9 (60)	9 (60)
B. Ampullary carcinoma Grade		EMA(%)	CEA(%)	P53(%)	C-neu(%)	TGF-a(%)
I	(n = 1)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)
II	(n = 2)	2 (100)	2 (100)	0 (0)	2 (100)	1 (50)
III	(n = 4)	4 (100)	2 (50)	0 (0)	4 (100)	2 (50)
IV	(n = 1)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
Total	(n = 8)	8 (100)	5 (63)	1 (13)	8 (100)	4 (50)

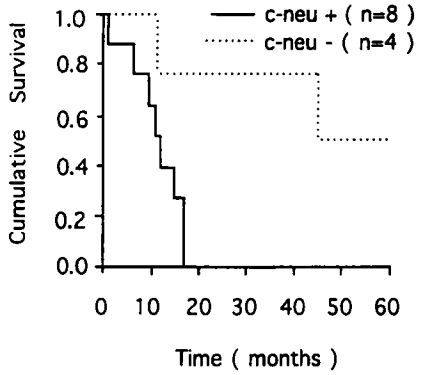

Fig. 2. Twelve patients with adenocarcinoma of duodenum underwent resection. All eight patients with c-neu positive tumors died within 18 months after resection, whereas only one with c-neu negative tumor died within 18 months. Patients with c-neu positive duodenal adenocarcinoma had a shorter survival than patients with c-neu negative duodenal adenocarcinoma ($P < 0.01$).

with poor prognosis in patients [23–25]. We found 60% of duodenal adenocarcinomas and 100% of ampullary adenocarcinomas to be c-neu positive; also the patients with c-neu positive duodenal adenocarcinoma had a shorter survival than patients with c-neu negative tumors. Compared with a previous study of six ampullary adenocarcinomas, of which 33% were c-neu positive [23], the present study had a higher positive rate. This might be due to the use of a different antibody or to the small group of patients. The positive staining pattern of c-neu is not only located at the cytoplasmic membrane of malignant cells, but also in the cytoplasm. This has been studied previously in normal pancreatic tissue and pancreatic cancer [18,23,26–28], normal breast tissue and breast cancer [19]. De Potter et al. [19] demonstrated that the cytoplasm staining noted by light microscopy is due to the binding of antibody to the inner mitochondrial membranes as visualized by electron microscopy. The function of this protein located on mitochondrial membranes is unknown.

C-neu overexpression has been shown to be associated with a high proliferative rate in breast cancer [29]. Ki-67, a nuclear antigen expressed by proliferating, cells, but not resting cells, can be detected by the MIB-1 monoclonal antibody. Utilizing this immunostaining method, Kearns and associates [30] observed a strong correlation between the Ki-67 (MIB-1) proliferative index and the overexpression of c-neu oncogene in advanced ovarian carcinomas. They also noted a shorter survival time among these patients with the higher proliferative indices. Study of such a proliferative marker might add another prognostic indicator for the duodenal and ampullary cancers under investigation.

Growth factors (GF) have been shown to play a role not only in the development of normal tissues, wound healing, hematopoietic cell proliferation, and differentiation, but also in the progression of cancer. Sporn and Todaro [31] and subsequently others [32] have proposed an autocrine hypothesis that certain tumors may produce their own growth factors and thereby proliferate independently of the host's systemic *resources*. TGF-α can stimulate epidermal cell proliferation by binding to the EGF receptor and its expression has been documented in several types of normal and neoplastic tissues [33–35]. We found that 60% of duodenal and 50% of ampullary adenocarcinomas were positive for TGF-α, but the present study did not show a significant relationship between the expression of TGF-α and the patients' survival, tumor stage or histological grade.

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